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(54) Title: METHOD FOR DELIVERING BIOLOGICALLY ACTIVE MATERIALS USING A THIOESTER OR THIOETHER PRODRUG

(57) Abstract

A method of delivery of biologically active compounds through biological membranes is provided that includes the attachment of the active compound via a thioester or thioether linkage to an inert compound, a secondary biologically active compound or a targeting compound, that facilitates transport to or through the membrane.

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Method for Delivering Biologically Active Materials Using a Thioester or Thioether Prodrug

This application is in the area of methods and compositions for the delivery of biologically active molecules.

Background of the Invention

There are a number of biological membranes, including those of the skin, the eye, the blood-brain barrier, and around the cell nuclei (i.e., the eukaryotic nuclear membrane or envelope), that act as natural barriers to chemical compounds. These membranes serve a vital role in protecting sensitive regions of the body from classes of compounds that might adversely affect the function of the protected region. However, these protective biological membranes also render it difficult to deliver pharmaceutical agents to regions of the body in need of medical therapy.

In order to pass through biological barrier membranes, the molecule must possess certain physical and chemical characteristics. Most notably, the molecule must be soluble in a non-polar (lipophilic) environment. A certain degree of water solubility is required, however, for a drug to be transported via the plasma through the membrane to the brain or eye or to get to the lower skin layers from the surface of the skin. For example, drugs must be substantially lipid soluble, to pass through the cornea for treatment of the eye, however, it may also be advantageous for the drug to have a degree of water solubility to allow passage through the aqueous humor or partition from the corneal epithelium to the vitreous body.

Skin

The skin is a complex organ which serves a number of important functions. The barrier function of the skin resides in the outermost layer, the stratum corneum. The stratum corneum is composed of lipid-rich "mortar" (containing cholesterol, free fatty acids and other components), and protein-rich "bricks" (processed epidermal cell remnants). The cutaneous barrier protects the body from infection, water and electrolyte loss, and the entry of toxic substances. Below the stratum corneum is the epidermis. The living epidermis continuously replenishes the components of the stratum corneum and provides another level of barrier protection. It is also the site where many immune responses against infectious agents and allergens from the environment are first induced. The dermis is the next layer of skin and is composed of a complex maze of connective tissue, blood vessels, nerves, and supporting cells. The healthy dermis is the scaffold which supports a supple, smooth cutaneous surface. The skin is also composed of a number of appendages, such as hair follicles, sweat glands, and sebaceous glands.

Treatment of skin disorders is currently most often accomplished by both systemic (oral) administration of drugs and topical application of drugs. The current therapies for many of these diseases are ineffective primarily because of the difficulty of delivering the drugs to the diseased skin site, even when administered topically. A more efficient means of transporting compounds to treat these disorders would result in greater benefit to the host and simplified administration.

The Eye

The anatomy of the eye of a vertebrate animal is protected from the outside by the cornea and from the inside by the sclera. Both the cornea and sclera serve to protect the retina and lens, which are responsible for the first steps in the visual process, i.e. the light induced chemical reactions which send the signals to the brain.

The cornea is the main refracting surface of the eye, provides a lipophilic layer that imparts protection from, and can be vulnerable to, environmental attacks, including exposure (direct trauma, drying, radiant and ionizing energy), infectious agents (bacteria, viruses - notably herpes simplex and herpes zoster - fungi, and parasites), and inflammation, sometimes in association with systemic dermatologic disorders such as atopic dermatitis, cicatricial pemphigoid, and erythema multiforme (Stevens-Johnson syndrome). While the cornea directly protects the iris and lens from assaults, it also prevents or minimizes the ability of potentially beneficial drugs for the treatment of ocular disorders or damage to enter the eye.

Like the blood-drain barrier, the sclera serves to protect the eye from the agents that enter into the bloodstream. This internal barrier prevents not only harmful agents from entering the eye, but also potentially therapeutic agents such as drugs, which are administered for the treatment of eye diseases or other eye damage.

There are a number of ocular disorders, including bacterial, viral, fungal infections, and pathogenic oxidation processes, that could be greatly alleviated by the administration of a therapeutic agent with an appropriate penetration profile. Pathogenic oxidation processes can cause

significant damage to the eye. The aqueous humor of the eye includes a significant amount of hydrogen peroxide. The anterior tissues bathed by the aqueous humor, including the cornea and the anterior portion of the lens, therefore exist in an oxidative environment, and can be harmed when the hydrogen peroxide level is above normal. Exposure of the eye to light of certain wavelengths can also cause harm to anterior, posterior and other tissues of the eye, including the lens, retina and retinal pigmented epithelium. Pathogenic ocular oxidation pathways can also be triggered in association with ischemia, a variety of drugs or endogenous cell regulators, or by pressure on tissues caused by pressure changes in the anterior chamber of the eye. Oxidative ocular processes are specifically involved in age-related cataracts, light-induced retinal damage, other retinopathies such as diabetic retinopathy or age-related macular degeneration, inflammatory damage, vascular leakage and edema (as in cystoid macular edema), accidental or surgical trauma, angiogenesis, corneal opacities, retrolental fibroplasia, and some forms of glaucoma.

Keratitis is an inflammation or infection of the cornea. It is often associated with inflammation of the iris (iritis) or of the uveal tract - the iris, ciliary body, and choroid (uveitis). Keratitis combined with uveitis or iritis is seen commonly in Reiter's disease and occasionally in Behcet's disease. Keratitis and uveitis may also occur with herpes simplex infection, in sarcoidosis, and in collagen vascular diseases. Metabolic disorders can also cause opacification of the cornea since the cornea can store material present in excess in the body. In hypercalcemia secondary to sarcoid, hyper-

parathyroidism, and vitamin D intoxication, calcium phosphates and carbonates precipitate beneath the corneal epithelium in a plane corresponding to the interpalpebral fissure, so-called band keratopathy. Cystine crystals are deposited in cystinosis, cholesterol esters in hypercholesterolemia (arcus senilis), chloroquine crystals in treatment of discoid lupus, polysaccharides in Hurler's disease, and copper in hepatolenticular degeneration (Kayser-Fleischer ring). Keratoplasty (transplantation) for restoration of sight can be warranted when the cornea is sufficiently scarred and/or opaque.

Blood-Brain Barrier

A major problem in treating traumatic injury and disorders of the central nervous system is the presence of the blood-brain barrier (BBB) which imposes limitations on the transportability of potential therapeutic agents from the plasma to the extracellular fluids of the brain and spinal cord. To traverse the CNS vasculature, a compound must first leave the plasma. The compound can then enter the capillary endothelial cell membrane, leave the membrane and enter the cytoplasm. Finally, the compound is conveyed through the outer cell membrane to the surrounding extracellular fluid in the brain. An alternative pathway for compound transport is via the inter-endothelial cell junction.

As with the eye, there are a number of disorders that affect the central nervous system that could be greatly alleviated by the administration of a therapeutic agent with an appropriate penetration profile. For example, oxygen radicals are known to be formed after traumatic CNS injury, and during reperfusion

following obstruction of CNS blood flow. One important mechanism by which free radicals are formed in the CNS immediately following experimental injury probably involves the cyclo-oxygenase metabolism of arachadonic acid. Accordingly, drugs that can scavenge free radicals before, during or after CNS traumatic injury may minimize the extent of the neurological damage which occurs in these settings.

Degenerative central nervous system disorders have also been linked to pathogenic oxidation processes. The cause of Parkinson's disease (PD), an example of a degenerative central nervous system disorder, is currently thought to be progressive damage to neurons by one or more environmental toxins. The major visible symptoms of Parkinson's Disease (PD) are tremor and bradykinesia (slow movement).

Alzheimer's disease is another example of a degenerative brain disease that is caused by a progressive and selective degeneration of neuron populations in discrete portions of the brain. It is manifested clinically by an impairment of memory and decision-making that begins insidiously and progressively worsens. There is no cure for Alzheimer's disease and no drug tried so far can alter the progress of the disease (Cecil Textbook of Medicine, 19th ed., Wyngaarden, J.B., Smith L.H., Bennett, J.C. Eds., Saunders: 1992, p. 2075).

Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's Disease) is a fatal degenerative disease of the CNS. In the case of ALS, degeneration of the motor neurons of the spinal cord and the brain stem causes slow progressive paralysis of the voluntary muscles. There is also an inflammatory component to the pathology of the disease. (Cecil

Textbook of Medicine, 19th ed., Wyngaarden, J.B., Smith L.H., Bennett, J.C. Eds., Saunders: Philadelphia, 1992, p. 2141).

Cell Nuclei

The nuclear envelope encloses the deoxyribonucleic acid (DNA) and defines the nuclear compartment. It is formed from two concentric membranes. The spherical inner nuclear membrane contains specific proteins that act as binding sites for the *nuclear lamina* that support it and its contacts with the chromosomes and nuclear ribonucleic acids (RNA). This membrane is surrounded by the outer nuclear membrane, which closely resembles the membrane of the endoplasmic reticulum.

The nucleus contains many proteins that help mediate its unique function. These proteins, which include histones, DNA and RNA polymerases, gene regulatory proteins, and RNA-processing proteins, are imported from the cytosol, where they are made. They must pass through both the outer and inner nuclear membranes to reach the inside of the nucleus (the nuclear lumen). This transport process is selective, many proteins made in the cytosol are excluded from the nucleus. In fact, the nuclear envelope seems designed to shield the contents of the nuclear compartment (the nucleoplasm) from many of the particles, filaments, and large molecules that function in the cytoplasm. Mature cytoplasmic ribosomes, for example, are too large to pass through the 9-nanometer channels of the nuclear membrane, insuring that all protein synthesis is carried out in the cytoplasm. Evidence exists that nuclear proteins are actively transported into the nucleus via receptor proteins.

The same properties of the nuclear

envelope that exclude normally-occurring cytoplasmic material from the nucleus render it difficult to administer therapeutic agents to the nucleus. For example, a number of technologies have been or are in the process of being developed to modulate gene expression, which occurs in the cell nucleus. One such technology is Antisense Oligonucleotide Technology (AOT), a therapy for cancer as well as other diseases. See, for example, Uhlmann, "Antisense Oligonucleotides: A New Therapeutic Approach" *Chemical Reviews*, 90(4), June 1990. Antisense technology refers in general to the modulation of gene expression through a process wherein a synthetic oligonucleotide is hybridized to a complementary nucleic acid sequence to inhibit transcription or replication (if the target sequence is DNA), inhibit translation (if the target sequence is RNA) or to inhibit processing (if the target sequence is pre-RNA). A wide variety of cellular activities can be modulated using this technique. A simple example is the inhibition of protein biosynthesis by an antisense oligonucleotide bound to mRNA. In another embodiment, a synthetic oligonucleotide is hybridized to a specific gene sequence in double stranded DNA, forming a triple stranded complex (triplex) that inhibits the expression of that gene sequence. Antisense oligonucleotides can also be used to activate gene expression indirectly by suppressing the biosynthesis of a natural repressor or directly by reducing termination of transcription. AOT can be used to inhibit the expression of pathogenic genes, for example, those that facilitate the replication of viruses, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and herpesviruses, and cancers, particularly solid tumor masses such as

gliomas, breast cancer, and melanomas.

It would be very useful to have a method to deliver biologically active agents for pharmaceutical purposes, through biological barrier membranes, including those in the skin, the eye, the blood-brain barrier, the nuclear membranes and those surrounding the cell nuclei.

Therefore, it is an object of the present invention to provide a method for the delivery of biologically active molecules through biological membranes, including those of the skin, eye, blood-brain barrier, cell membranes and cell nuclei.

It is another object of the present invention to provide modified biologically active molecules that are capable of passing through barrier biological membranes and which may possess, or can be restored to, an active state after passing through the membrane.

It is a further object of the present invention to provide pharmaceutical compositions that include modified biologically active molecules that are capable of passing through barrier biological membranes and which can be restored to an active state (if necessary) after passing through the membrane.

Summary of the Invention

A method is presented for the modification of biologically active molecules that are useful in the treatment of disorders but which have physical characteristics that limit their ability to cross a biological membrane. The modification is reversed after passing through the barrier membrane, allowing the restoration of the molecule to its active state.

In one embodiment, this method includes

linking a biologically active molecule to a lipophilic tail through a thioester or thioether bond (linkage). The thioester linkage is stable in nonbiological aqueous fluids such as intravenous fluids, sterile saline, and other water-based pharmaceutical carriers such as creams and ointments, for an extended period. When placed in biological fluids, the thioester bond is broken down to release the biologically active molecule and the inert, biologically compatible carrier. In another embodiment, the biologically active molecule is linked to a second biologically active molecule through a thioester or thioether bond (linkage). The activities of the two biologically active compounds can be synergistic or independent.

In an alternative embodiment, a method is provided for the delivery of a biologically active molecule to a specific site, for example, a plasma membrane surface or a nucleus of a particular cell type. In this embodiment, a biologically active molecule is linked to a second "targeting molecule" through a thioester or thioether bond. In a preferred embodiment, the targeting molecule is a moiety that binds to a receptor molecule on the target membrane's surface. Examples of targeting molecules include steroids (including corticosteroids, androgens, estrogens, progestins, mineralocorticoids), or within the cytosol retinoids (including retinoic acid, etretinate, isotretinoin etretin), hormones (including but not limited to melatonin, thyroid stimulating hormone, and gastrin), hormone receptors, cell specific receptors and ligands that bind to cell specific receptors (including but not limited to sugars, proteins, peptides, and glycoproteins), antibodies, antibody fragments (such as the Fab or Fab_2 antibody fragments), antigens, T-cell receptor fragments

including T-cell receptor variable regions and cytokine receptors (including but not limited to IL1, IL2, IL4, IL6, TNF-α and TGF-ß), and growth factor receptors (including but not limited to Basic Fibroblast Growth Factor, Acidic Fibroblast Growth Factor, Epidermal Growth Factor, and TGF-α). In one embodiment, a steroid that binds to the membrane of a cancer cell or intracytoplasmic receptor of a cancer cell, is used as the targeting moiety.

In another embodiment, the biologically active molecule is linked to a lipophilic tail, second biologically active molecule, or targeting compound through a spacer that includes one or more thioester or thioether linkages. The spacer can be formed, for example, from a peptide, protein or aliphatic carbon chain with appropriate functional groups.

Detailed Description of the Invention

A general method of delivery of biologically active compounds through biological membranes is provided that includes the attachment of the active compound via a thioester or thioether linkage to either a biologically inert compound or a secondary biologically active compound that facilitates transport to or through the membrane. In one embodiment, the modified biologically active molecule is designed to pass through biological membranes such as the skin (i.e., for dermatological applications), the blood-brain barrier (i.e., for treatment of central nervous system disorders), the eye (i.e., for ophthalmic applications), or the cell nucleus (i.e., for gene therapy), or for the treatment of hyperproliferating, hyperplastic, or malignant

cells.

In one embodiment, a method for the delivery of a biologically active agent is provided that includes administering an effective amount of the biologically active agent in the form of a compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R' is a moiety that facilitates transport through or targets a specific membrane. In a second embodiment, a method for the delivery of a biologically active agent is provided that includes administering an effective amount of the biologically active agent in the form of R-S-R'. In another embodiment, the biologically active molecule is linked to a lipophilic tail, second biologically active molecule, or targeting compound through a spacer that includes one or more thioester or thioether linkages. The spacer can be formed, for example, from a peptide, protein or aliphatic carbon chain with appropriate functional groups.

It has been discovered that thioester linkages are particularly useful because they are relatively stable in non-biological aqueous or organic preparations, yet they readily hydrolyze or are enzymatically degraded in the presence of biological fluid components. For example, hydrolysis of the thioester linkage occurs in aqueous solutions with added fetal calf serum, in conditioned media harvested from mouse keratinocyte cell cultures, and in aqueous solutions with small pieces of human skin added.

The hydrolysis or enzymatic breakdown products of the prodrug, RC(O)OH, R'SH, RSH, or R"C(O)OH, as appropriate, should be selected to be biocompatible in the selected host, typically a mammal and in particular a human. The term

biocompatible refers to a material that is substantially nontoxic to the host in the range of dosages administered.

I. Biologically Active Molecules to be Delivered

The term biologically active molecule or material as used herein refers to an organic molecule including a drug, a protein, polysaccharide, nucleoprotein, lipoprotein, synthetic polypeptide, or a small molecule linked to a protein, carbohydrate, glycoprotein, steroid, nucleic acid, lipid, or combination thereof, that causes a biological effect when administered to an animal, including but not limited to birds and mammals, including humans. The term drug, as used herein, refers to any substance used systemically or topically for the treatment, cure, or prevention of a disease or disorder.

Drugs that can be delivered using this method include but are not limited to immunosuppressants, antioxidants, anesthetics, chemotherapeutic agents, steroids (including retinoids), hormones, antibiotics, antivirals, antifungals, antiproliferatives, antihistamines, anticoagulants, antiphotoaging agents, melanotropic peptides, and nonsteroidal and steroidal anti-inflammatory compounds. Examples of other molecules that can be delivered include nucleosides, nucleotides, oligonucleotides (including antisense oligonucleotides), cDNA, nucleic acids, and genes. Vitamins, including vitamin C, vitamin D (including vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, 1α,25-dihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3), vitamin E, and their derivatives can also be administered using this delivery technology.

Specific biologically active agents include calcipotriene, N-acetylcysteine, nonsteroidal antiinflammatories including acetaminophen, indomethacin, ibuprofen, salicylic acid and derivatives, glucocorticoids and other steroidal anti-inflammatories, antibiotics including erythromycin, derivatives of erythromycin, tetracycline, derivatives of tetracycline, metronidazole and minoxidil.

Of special interest are agents for the treatment of dermatological disorders, ocular (including corneal) disorders, and central nervous system disorders and cancer. Using the method disclosed herein, pharmaceutically active agents for these disorders, including but not limited to those listed below, can be delivered in a manner that allows the active agent to penetrate the relevant barrier biological membrane.

A. Dermatological Disorders

Current agents for the treatment of dermatological disorders that can be delivered using this method include, but are not limited to acitretin (etretin), minoxidil, dithranol (anthralin, dioxyanthranol), etrinate, vitamin A, vitamin A derivatives, isotretinoin, pyrogallol (pyrogallic acid), resorcinol, and salicylic acid.

B. Ocular Disorders

Agents for the treatment for a range of disorders, including ocular disorders that can be delivered using this method include, but are not limited to bacitracin zinc, chloramphenicol, chlortetracycline, hydrochloride, ciprofloxacin hydrochloride, erythromycin, gentamycin sulfate, norfloxacin, sufacetamide sodium, sulfisoxazole diolamine, tetracycline hydrochloride, tobramycin

sulfate, polymyxin B, neomycin gramicidin, oxytetracycline, trimethoprim, amikacin, vancomycin, cefazolin, tramcinolone diacetate, cefazolin, prednisolone acetate, amikacin sulfate, ampicillin sodium, bacitracin zinc, carbenicillin disodium, cefazolin sodium, ceptazidime, clindamycin, colistimethate sodium, erythromycin, gentamycin sulfate, imipenem, cilastatin sodium, kanamycin sulfate, methicillin sodium, neomycin sulfate, penicillin G, polymyxin B sulfate, ticarcillin disodium, tobramycin sulfate, vancomycin hydrochloride, amphotericin B, flucytosine, fluconazole, natamycin, miconazole nitrate, ketoconazole, idoxuridine, trifluridine, vidarabine, acyclovir, foscarnet, ganciclovir, dexamethasone, dexamethasone sodium phosphate, fluorometholone, fluorometholone acetate, medrysone, prednisolone acetate, prednisolone sodium phosphate, diclofenac, flubiprofen, ketorolac, suprofen, naphazoline hydrochloride, phenylephrine hydrochloride, and tetrahydrozoline hydrochloride.

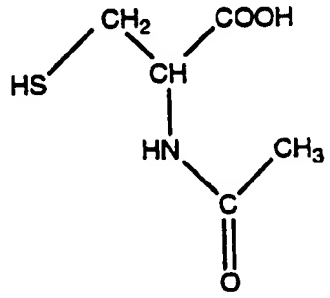
C. N-Acetyl Cysteine and Derivatives Thereof, Antioxidants

Cysteine is an amino acid with one chiral carbon atom. It exists as an L-enantiomer, a D-enantiomer, or a racemic mixture of the L and D enantiomers. The L-enantiomer is the naturally occurring configuration.

N-acetylcysteine (acetamido-mercaptopropionic acid, NAC) is the N-acetylated derivative of cysteine, as illustrated below. It also exists as an L-enantiomer, a D-enantiomer, an enantiomerically enriched composition of one of the enantiomers, or a racemic mixture of the L and D enantiomers. The term "enantiomerically enriched composition or compound" refers to a composition or

compound that includes at least 95%, and preferably, at least 97% by weight of a single enantiomer of the compound. Any of these forms of NAC can be delivered for the treatment of a variety of disorders.

In a preferred embodiment, a single isomer of a thioester or thioether of NAC or its salt, and most preferably, the naturally occurring L-enantiomer, is used in the treatment process.



N-acetylcysteine exhibits antioxidant activity (Smilkstein, Knapp, Kulig and Rumack, *N. Engl. J. Med.* 1988, Vol. 319, pp. 1557-62; Knight, K.R., MacPhadyen, K., Lepore, D.A., Kuwata, N., Eadie, P.A., O'Brien, B. *Clinical Sci.*, 1991, Vol. 81, pp. 31-36; Ellis, E.F., Dodson, L.Y., Police, R.J., *J. Neurosurg.*, 1991, Vol. 75, pp. 774-779). The sulfhydryl functional group is a well characterized, highly reactive free radical scavenger. N-acetylcysteine is known to promote the formation of glutathione (a tri-peptide, also known as glutamylcysteinylglycine), which is important in maintaining cellular constituents in the reduced state (Berggren, M., Dawson, J., Moldeus, P. *FEBS Lett.*, 1984, Vol. 176, pp. 189-192). The formation of glutathione may enhance the activity of glutathione peroxidase, an enzyme which inactivates hydrogen peroxide, a known precursor to hydroxyl radicals (Lalitha, T., Kerem, D., Yanni, S., *Pharmacology and Toxicology*, 1990, Vol.66, pp. 56-61).

Other known activities of N-acetylcysteine include its effectiveness as a mucolytic agent, wherein the pharmacology is related to the reactive sulfhydryl group in the molecule (Lightowler and Lightowler, *Arch. Int. Pharmacodyn. Ther.* 1971, Vol. 189, pp. 53-8). The sulfhydryl group probably opens sulfide linkages in mucus, thereby lowering mucosal viscosity. NAC is also used for the treatment of acetaminophen overdoses (Smilkstein, Knapp, Kulig and Rumack, *N. Engl. J. Med.* 1988, Vol. 319, pp. 1557-62). A large overdose of acetaminophen results in a larger portion of the drug being metabolized via a free radical (cytochrome P-450) pathway which results in hepatic cellular necrosis. N-acetylcysteine, when administered within the first few hours of overdose, protects the liver by acting as an alternate sub. rate for conjugation with, and detoxification of, the reactive metabolite.

In addition to its mucolytic and free radical scavenging ability, NAC has been reported to be an effective collagenase inhibitor (Lemp and Roddy, Ann. Ophthalmol. 1974, Vol. 6, pp. 893-5). It has also been reported that NAC reduces the activity of the proteolytic porcine enzymes, leukocyte elastase and pancreatic elastase, by greater than 55% *in vitro* (Morrison, Burnett and Stockley, *Biol. Chem. Hoppe Seyler* 1986, Vol. 367, pp. 177-82). In yet another capacity, N-acetylcysteine can act as an inhibitor of tumor necrosis factor-alpha (TNF-α) activity *in vivo* (Peristeris, P. et al, *Cell. Immunol.* 1992, Vol. 140, pp. 390-99).

It has been discovered that thioester and thioether prodrugs of N-acetylcysteine can be used to treat a wide variety of disorders. For example, traumatic injuries and acute or chronic disorders

of the central nervous system and the eye, including the retina and optic nerve, that are mediated by pathogenic oxidation processes can be treated by the administration of an effective amount of a lipid soluble thioester or thioether of N-acetylcysteine. In the preferred method of administration, the active compound or its pharmaceutically acceptable salt are administered in a suitable carrier for CNS or ophthalmic delivery.

N-acetylcysteine acts as an antioxidant that minimizes the injurious effect of the pathogenic oxidizing species in the skin, CNS or eye. It has been discovered that although N-acetylcysteine itself is not an effective drug for the treatment of CNS, ocular oxidative injury and disorders or topical treatment for skin disorders because it is not sufficiently lipophilic to be absorbed into the appropriate regions, when derivatized as a lipophilic thioester or thioether as illustrated above, it passes into the skin, CNS or eye, as desired. Therefore, the compounds described herein represent a new prodrug form of NAC for the efficient delivery of NAC to otherwise inaccessible regions of the body. Further, it has been found that thioester derivatives of NAC are stable in non-biological aqueous solutions such as saline, phosphate-buffered saline, and water-containing creams, however, in biological fluids such as plasma and tissue the derivative is converted to the parent N-acetylcysteine that exhibits the desired antioxidant properties.

N-acetylcysteine exhibits low toxicity in vivo, and is significantly less toxic than deprenyl (for example, the LD_{50} in rats has been measured at 1140 and 81 mg/kg intravenously, for N-acetylcysteine and deprenyl, respectively).

The delivery of N-acetylcysteine through the blood-brain barrier in the form of a thioester or thioether is useful in the treatment of symptoms associated with Parkinson's Disease, Alzheimer's Disease, amyotrophic lateral sclerosis, acute traumatic spinal cord and brain injury, reperfusion injury or other degenerative diseases of the CNS, or in the treatment of CNS trauma.

The thioester or thioether of NAC is also useful in the treatment, minimization, or prevention of a wide variety of ocular injuries and disorders that are mediated by pathogenic oxidative processes, including but not limited to macular degeneration, optic neuritis, retrobulbar neuritis, inherited optic atrophies, diabetic retinopathy, cataract formation, glaucoma or the risk of glaucoma associated with significantly elevated intraocular pressure, inflammatory eye disease, retinal eye disease, intraocular pressure rise due to uveitis, post-infarct amblus, traumatic eye injury (such as blunt trauma, compression injury, hyphema, surgical trauma, etc.), neovascular or ischemic eye disease (condition in the eye involving ischemia such as corneal edema from prolonged wearing of contact lenses and the like), bullous keratitis, dry eye including kerato-conjunctivitis sicca and alkali burn and conditions arising from transplantation of a corneal graft or transplantation of ocular cells.

A method and composition for the treatment of a cutaneous, ocular, or mucosal condition in a human or other mammal resulting from pathology associated with an immune response or inflammatory condition is also provided that includes the topical or systemic administration of an effective amount of a lipid soluble thioester or thioether of a biologically active compound, a

derivative thereof or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for topical or systemic administration. The thioester and thioether derivatives of the biologically active compound are administered as a general immunosuppressive and/or anti-inflammatory agent. The compounds may be useful as specific topical or systemic agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, aphthous ulcers, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The active compounds may also be useful in reducing the infiltration of skin by malignant leukocytes in diseases such as mycosis fungoides. These compounds may also be effective to treat an aqueous-deficient dry eye state (such as immune mediated keratoconjunctivitis) in a patient suffering therefrom, by administering the compound topically to the eye.

Prodrugs of N-acetylcysteine are also useful in the treatment of a cutaneous condition characterized by hyperkeratosis in a human or other mammal. In addition, the inflammatory condition associated with many of these diseases may also be alleviated with the administration of thioester derivatives of N-acetylcysteine, or its salts. A method of treatment is provided that includes the topical application of an effective amount of thioester derivatives of N-acetylcysteine or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for

topical application.

Thioester derivatives of N-acetylcysteine are administered as general treatments for hyperkeratotic disorders and/or as anti-inflammatory agents. The compounds may be useful as specific topical agents in treating cutaneous disorders characterized by hyperkeratosis including ichthyosis, keratoderma, lichen planus, and psoriasis.

Finally, a method for the topical or systemic treatment of disorders mediated by proteases that result in skin or mucosal lesions, and in particular, pemphigus, cicatricial pemphigoid, bullous pemphigoid, lichen planus, and canker sores (aphthous ulcers), is disclosed wherein the host is treated with an effective amount of a thioester or thioether derivative of N-acetylcysteine, or its pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable diluent or carrier for systemic or topical delivery. The active compound or its derivative is administered for a sufficient time period to alleviate the undesired symptoms and or the clinical signs associated with the disorder. Oral lesions associated with these disorders can be treated, for example, with an mouthwash rinse that contains an effective amount of N-acetylcysteine or its derivative or salt. The mouthwash is used as often as necessary to obtain amelioration of symptoms, and typically from one to several times a day until the desired benefit is achieved. The rinse is swished and expectorated or swallowed by the patient.

D. Antisense Therapy

Lipophilic oligonucleotide prodrugs of the present invention can be used to deliver a

particular oligonucleotide to a cell nucleus. Additionally, the antisense oligonucleotide can be coupled to steroid molecules either directly or indirectly to affect targeted delivery to the nucleus. Oligonucleotides that are capable of binding to polyribonucleic acid or polydeoxyribonucleic acid are useful as antisense agents. See generally, Antisense Molecular Biology and S-oligos, *Synthesis* 1 (Oct. 1988) (published by Synthecell Corp., Rockville, Md.); 2 Discoveries in Antisense Nucleic Acids (C. Brakel and R. Fraley eds. 1989); Uhlmann, et. al., "Antisense Oligonucleotides: A New Therapeutic Technique," *Chem. Rev.* **90(4)**, 1990; and Milligan, J.F., Matteucci, M.D., Martin, J.C., J. Med. Chem., 1993, 36, 1923-1937, all of which are incorporated herein by reference.

Antisense agents should be selected that are capable of selectively binding to a predetermined polydeoxyribonucleic acid sequence or polyribonucleic acid sequence or to a cell containing such sequence (e.g., by adding the antisense agent to a culture medium containing the cell) so that the antisense agent is transported through the cell membrane, binds to the predetermined sequence, and blocks transcription, translation, or replication thereof. The require-ments for selective binding of the antisense agent are known (e.g., a length of up to approxi-mately 17 bases for selective binding within the human genome).

II. Biologically Active Molecule Linked to Lipophobic Tail

In one embodiment, a method and composition is provided for the delivery of a biologically active agent through a biological membrane that includes the biologically active

agent in the form of a compound selected from the group consisting of R-C(O)S-R', R-SC(O)-R', or R-S-R', wherein R is the residue of the biologically active agent and R'- is a lipophilic moiety which ennances the overall lipophilicity of the prodrug.

As used herein, the term lipophilic refers to a material that is more soluble in a nonpolar organic solvent such as octanol than in water. This can be evaluated conveniently by determination of the material's octanol water partition coefficient. In general, it is desirable that the lipophilic prodrugs disclosed herein have an octanol water partition coefficient of 1.0 or greater (optimally at least 2, 5, 10 or greater). In one embodiment, a lipophilic thioester or thioether prodrug is provided in which the prodrug has a partition coefficient which is at least 2, and preferably 5 or 10 or more, times greater than the partition coefficient of the parent biologically active molecule.

R' can be selected from the group consisting of alkyl, alkenyl, alkynyl, alkyaryl, aralkyl, haloalkyl, haloalkenyl, haloalkynyl, -C_{1-10}alkyl(oxy)C_{1-10}alkyl, -C_{1-10}alkyl(thio)C_{1-10}alkyl, aryloxyalkyl, aryl, including but not limited to phenyl and benzyl, and heterocycles.

The term heteroaryl or heterocycle, as used herein, refers to an aromatic moiety that includes at least one sulfur, oxygen, or nitrogen in the aromatic ring. Nonlimiting examples are furyl, pyridyl, pyrimidyl, thienyl, isothiazolyl, imidazolyl, tetrazolyl, pyrazinyl, benzofuranyl, benzothiophenyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, isoindolyl, benzimidazolyl, purinyl, carbozolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,4-thiadiazolyl, isooxazolyl, pyrrolyl, pyrazolyl,

quinazolinyl, pyridazinyl, pyrazinyl, cinnolinyl, phthalazinyl, quinoxalinyl, xanthinyl, hypoxanthinyl, pteridinyl, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, pyrazolopyrimidinyl, adenine, N^6-alkylpurines, N^6-benzylpurine, N^6-halopurine, N^6-vinylpurine, N^6- acetylenic purine, N^6-acyl purine, N^6-hydroxyalkyl purine, N^6-thioalkyl purine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrmidine, uracil, N^5-alkylpyrimidines, N^5-benzylpyrimidines, N^5-halopyrimidines, N^5-vinylpyrimidine, N^5-acetylenic pyrimidine, N^5-acyl pyrimidine, N^5-hydroxyalkyl purine, and N^6-thioalkyl purine, and isoxazolyl. Functional oxygen and nitrogen groups on the heterocyclic base can be protected as necessary or desired during the reaction sequence. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, and t-butyldiphenylsilyl, tritylmethyl, alkyl groups, acyl groups such as acetyl and propionyl, methylsulfonyl, and p-toluylsulfonyl.

The term alkyl, as used herein, refers to a saturated straight, branched, or cyclic (or a combination thereof) hydrocarbon of C_1 to C_{22}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropylmethyl, cyclobutylmethyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, heptyl, octyl, nonyl, and decyl.

The term aryl, as used herein, refers to phenyl, or substituted phenyl, wherein the substituent is halo, alkyl, alkoxy, alkylthio, haloalkyl, hydroxyalkyl, alkoxyalkyl, methylenedioxy, cyano, C(O)(alkyl), carboxylic

acid, CO_2alkyl, CO_2aryl, amide, amino, alkylamino or dialkylamino, and wherein the aryl group can have up to 3 substituents.

The term alkenyl, as referred to herein, and unless otherwise specified, refers to a straight, branched, or cyclic (in the case of C_{5-6}) hydrocarbon of C_2 to C_{10} with at least one double bond.

The term alkynyl, as referred to herein, and unless otherwise specified, refers to a C_2 to C_{10} straight or branched hydrocarbon with at least one triple bond.

The term aralkyl refers to an aryl group with an alkyl substituent.

The term alkaryl refers to an alkyl group with an aryl substituent, including benzyl, substituted benzyl, phenethyl or substituted phenethyl, wherein the substituents are as defined above for aryl groups.

As used herein the term fatty acid refers to a long chain (C_6 to C_{24}) aliphatic carboxylic acid, including saturated and unsaturated acids.

The term halo, as used herein, includes fluoro, chloro, bromo, and iodo.

The term halo (alkyl, alkenyl, or alkynyl) refers to a (alkyl, alkenyl, or alkynyl) group in which at least one of the hydrogens in the group has been replaced with a halogen atom.

The term aralkyl refers to an aryl group with an alkyl substituent.

The term alkaryl refers to an alkyl group that has an aryl substituent.

In one embodiment, R'C(O)- is the residue of a saturated or unsaturated fatty acid. Nonlimiting examples of fatty acids are lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic,

palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic and petriselinic acid.

III. Biologically Active Molecule linked to Second Biologically Active Molecule

In an alternative embodiment, a biologically active molecule is delivered that is linked to a second biologically active molecule via a thioester or thioether linkage. In a preferred embodiment, the two biologically active agents exhibit complimentary activities, for example, the two agents are therapeutically useful for the same indication or for two or more indications that are present together in the host.

As nonlimiting examples, R' can be any of the biologically active moieties described in detail above, or can be the residue of a compound selected from the group consisting of vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, 1α,25-dihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3, vitamin E, and vitamin C. Alternatively, R or R' is selected from the group consisting of erythromycin, propionylerythromycin, neomycin, gentomycin, mechlocyclin, tobramycin, and kanamycin and other antibiotics, antifungals, antivirals, chemotherapeutic agents, and antisense oligonucleotides.

IV. Biologically Active Molecules Targeted to Specific Site

In another embodiment, a method is provided for the delivery of a biologically active molecule to a specific site, for example, a plasma membrane surface, cytosol, or a nucleus of a particular cell type. In this embodiment, a biologically active molecule is linked to a second "targeting molecule" through a thioester or thioether bond. This linkage can be either direct or indirect, and in the latter case the linker can be either a peptide, protein or aliphatic carbon chain with appropriate functional groups. In a preferred embodiment, the targeting molecule is a moiety that binds to a receptor molecule on the target membrane's surface, in the cytosol of a cell, or within the nucleus of a particular cell type. Examples of targeting molecules include steroids (including corticosteroids, androgens, estrogens, progestins, mineralocorticoids), or within the cytosol retinoids (including retinoic acid, etretinate, isotretinoin etretin), hormones (including but not limited to melatonin, thyroid stimulating hormone, and gastrin), hormone receptors, cell specific receptors and ligands that bind to cell specific receptors (including but not limited to sugars, proteins, peptides, and glycoproteins), antibodies, antibody fragments (such as the Fab or Fab_2 antibody fragments), antigens, T-cell receptor fragments including T-cell receptor variable regions and cytokine receptors (including but not limited to IL1, IL2, IL4, IL6, TNF-α and TGF-ß), and growth factor receptors (including but not limited to Basic Fibroblast Growth Factor, Acidic Fibroblast Growth Factor, Epidermal Growth Factor, TGF-α).

As an example, steroids can be used to

deliver chemotherapy to a variety of pathogenic cells. Examples include estrogens (for targeting certain benign or malignant tumors of the breast, ovary and endometrium) or testosterone (for targeting certain benign or malignant tumors of the prostate, testes, and for targeting sebaceous and pilosebaceous units); progesterones (for targeting certain benign or malignant tumors of the breast, ovary, and endometrium); and corticosteroids (lymphocytes, antigen presenting cells and other immune cells, as well as osteoblasts, etc.).

V. Synthetic Methods for the Modification of Biologically Active Molecules

The modification of biologically active molecules into thioester lipophilic prodrugs can be accomplished in a wide variety of ways known to those skilled in the art. Any of these known methods can be used to provide the compounds described herein. A general description of several of these techniques is presented below.

It is typically desirable as a first step to protect any functional groups on R or R' that are not involved in the coupling step and which may promote side reactions. The term "protected" refers to a group that is added to a functional group, typically an oxygen, nitrogen, or sulfur containing group, to prevent undesired side reactions during the course of derivatization of other moieties in the molecule in which the functional group is located. A wide variety of protecting groups are known to those skilled in the art of organic synthesis. See, generally, Greene and Wuts, Protecting Groups in Organic Chemistry, John Wiley (1991). These protecting groups can be removed during the appropriate synthetic step by techniques well known to those skilled in the art

of organic chemistry.

Formation of Thioester Linkages

In one embodiment, the formation of the compounds of the present invention involves formation of thioester linkages. These linkages can be generated by a wide variety of methods, including but not limited to: 1) reacting a thiol group with a carboxylic acid or a carboxylic acid derivative; 2) reacting a -C(O)SR synthon (synthetic equivalent) with a suitable electrophile; and 3) reacting a compound of the formula $RC(O)S^-$ with a suitable electrophile. It should be noted that the latter two reaction schemes involve S_N2 transformations that invert the stereochemistry at that carbon, and therefore, may not be appropriate in situations in which the stereochemical configuration at a modified location plays a role in the activity of the molecule. In a preferred embodiment, if an S_N2 or other reaction that changes a stereochemical configuration is used to create or attach a thioester linkage on a biologically active molecule, that reaction is carried out at a bulk tolerating region of the molecule that is distant from the portion of the molecule that is critical for biological activity. The effect of derivation at various locations of a molecule on the biological activity of that molecule can be determined by carrying out a classical structure activity relationship (SAR) evaluation, or through molecular modeling.

Methods available for activating a carboxyl group to facilitate coupling with a thiol group to form a thioester linkage are numerous, and range from traditional methods that include the use of intermediate acid chlorides, acid anhydrides, and acyl azide formations, to more sophisticated

and recent methods involving the use of mixed anhydrides and active esters. Representative methods are listed in Larock, Comprehensive Organic Transformation, VCH, New York, 966-972 (1989), hereby incorporated by reference.

Reactions of activated carboxylic acids with thiol groups should be carried out in aprotic solvents to avoid reaction of the derivative with the solvent. Preferred solvents include dialkyl formamides, such as dimethylformamide, dialkyl sulfoxides, such as dimethylsulfoxide, aromatic solvents such as benzene, toluene, and xylenes, chlorinated solvents such as dichloromethane, chloroform, and trichloroethane, and ethers such as tetrahydrofuran and dibutyl ether. Stoichiometric amounts of trialkyl amines, pyridine, or other aprotic bases as acid scavengers are often used to facilitate the reaction.

The use of *N-N'*-dicyclohexylcarbodiimide (DCC) and other carbodiimides as activation reagents in peptide synthesis is well known. This reagent can also be used to activate carboxylic acids. Examples of other dehydrating agents that can be used are described in March, J., Advanced Organic Chemistry, John Wiley & Sons, (1992), hereby incorporated by reference.

Thioester linkages can also be formed by dehydrating a selected RSH or R'SH and a selected $R'CO_2H$ or RCO_2H, appropriately protected as necessary. In a typical dehydration procedure, triethylamine is added to a mixture of the carboxylic acid, diethyl phosphorocyanidate (DEPC) or diphenyl phosphorazidate (DPPA), and the thiol in dimethylformamide (DMF). The reaction is typically performed under ambient conditions. Workup procedures typically include washing the mixture with a weak acid solution, then

neutralizing the acid with carbonate or bicarbonate.

Displacement of Leaving Groups with NaSH or Thiolates

A biologically active molecule containing or modified to contain a leaving group, including but not limited to epoxide, chloride, bromide, iodide, tosylate, mesitylate, or triflate, can be reacted with NaSH to provide a thiol. The resulting thiol can be reacted with an activated carboxylic acid or carboxylic acid derivative to form a thioester.

Since NaSH is somewhat basic, and in some cases can result in elimination products, it is often preferred to use sodium or potassium thioacetate as a nucleophile. Reaction of the resulting thioester with methanol with a catalytic amount of acid results in the formation of a thiol and methylacetate, which is readily distilled out of the reaction vessel as it is formed.

Alternatively, one can form the thioester directly by reacting a biologically active molecule that contains a $C(O)S^-$ group with a second molecule that contains a suitable leaving group.

Compounds of the formula (phenyl)SCH_2NO_2 can be reacted with KOH, and the resulting nucleophile reacted with an aldehyde of the formula RCHO or R'CHO. Reaction of the resulting intermediate with mesyl chloride, and subsequent oxidation with ozone, yields a thioester of the formula PhSC(O)R. Banks, B.J., *et al.*, J. Chem. Soc., Chem. Commun., 670 (1984). This reaction can be modified as appropriate to provide prodrugs with carrier groups other than phenyl.

Compounds of the formula $RSOCH_2Li$ or $RSOCH_2Na$ (or $R'SOCH_2Li$ or $R'SOCH_2Na$) can be reacted with aldehydes of the formula R'CHO (or RCHO,

respectfully), and the resulting intermediate reduced with MnO_2 to yield compounds of the formula RSC(O)R' (or R'SC(O)R). Iriuchijima, S., *et al.*, J. Am. Chem. Sec., 97:596 (1975).

Alternatively, a free (unprotected) hydroxy group on a sugar, peptide, or nucleotide can be converted to a thiol group by converting the hydroxyl group into a suitable leaving group, such as mesylate, triflate, tosylate, or other leaving groups known to those skilled in organic synthesis, and then reacting the leaving group with NaSH or a compound of the formula $R\text{-}C(O)S^-$. Reaction with NaSH can be preferred if the leaving group is primary. However, use of NaSH is often accompanied with formation of H_2S, which is highly toxic and smelly. Further, if the leaving group is secondary, unwanted elimination reactions can occur. For these reasons, it may be preferred to react the leaving group with a thioacetate, such as sodium or potassium thioacetate. The resulting thioester can be hydrolyzed, for example, by reacting the thioacetate with methanol and an acid catalyst. The resulting methyl thioacetate can be readily distilled from the thiol.

Compounds containing S^- groups can be oxidized to S-S groups under the conditions of reaction. This oxidation can be minimized by performing the reactions under a nitrogen atmosphere, and degassing the solvents prior to reaction. Unwanted disulfides can often be reduced to the corresponding thiols by reaction with dithiothreitol (DTT), by means known to those skilled in organic synthesis. This can be preferred when the thiol group is on an expensive biologically active material, where even a slight yield loss due to oxidation is undesired.

When the biologically active molecule is

a peptide or protein, the peptide chain length can be extended (preferably from the N-terminus) by the addition of a sulfhydryl containing amino acid or amino acid derivative (i.e., cysteine, N-acetylcysteine, etc.), whose free sulfhydryl group can then be used to form the thioester or thioether linkage.

Thioether Formation

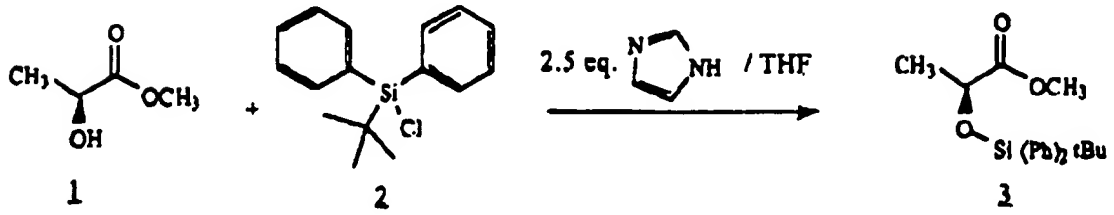
Thioethers can be formed in a variety of processes known to those skilled in the art. Any of these methods can be used to prepare the compounds described herein. As one example, a thiolate anion (e.g., RS^- or $R'S^-$) can be reacted with a biologically active molecule or carrier that contains a suitable leaving group, in a variation of the Williamson reaction. Suitable leaving groups include, but are not limited to chloride, bromide, iodide, tosylate, mesylate and triflate. Preferred solvents for the reaction include polar, aprotic solvents, including but not limited to dimethyl sulfoxide, dimethyl formamide, glyme, diglyme, tetrahydrofuran, acetone, methyl ethyl ketone, dibutyl ether and diethyl ether. Importantly, thiolates can be readily oxidized to disulfides at elevated temperatures in the presence of oxygen. Care must be taken to keep oxygen out of the reaction in order to avoid unwanted disulfide products.

Methods for the preparation of thioester prodrugs are more clearly understood by reference to the following detailed examples. These examples are merely illustrative, and are not intended to limit the scope of the invention.

Example 1 Preparation of N-Ac tyl-S-(L)-lactoyl-(L)-cysteine

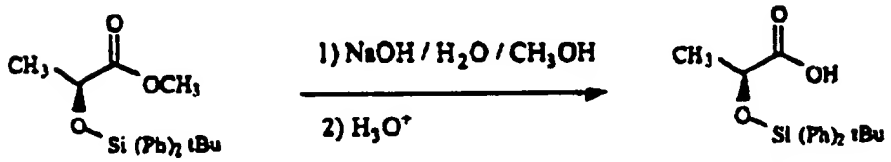
Synthetic route:

Step 1: Protection of the hydroxyl function of the lactic acid moiety



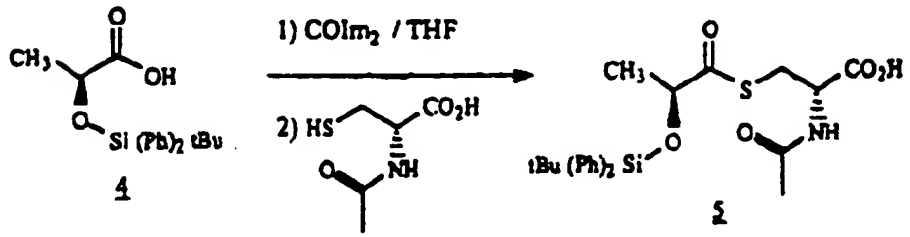
1 2 3

Step 2: Hydrolysis of methyl ester of 3



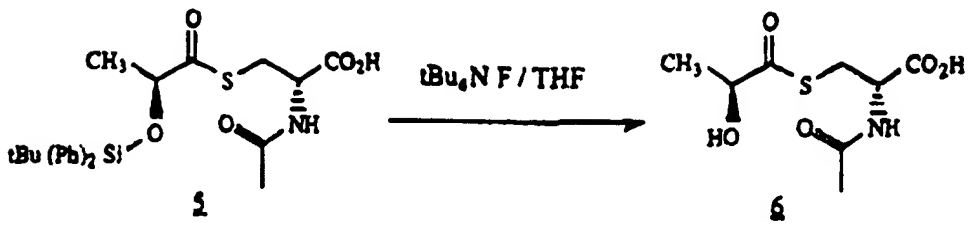
3 4

Step 3: Activation of the carboxylic acid and coupling with N-acetyl-(L)-cysteine



4 5

Step 4: Deprotection of the hydroxyl group



5 6

Synthesis of O-(t-butyldiphenylsilyl-methyl-(L)-lactate (3)

Methyl-(*S*)-(-)-lactate, 1 (0.50 mol, 51.6 g) and imidazole (1.1 mol, 74.4 g) were dissolved in 60 Ml of anhydrous tetrahydrofuran (THF). The reaction mixture was stirred under N_2 for 10 to 15 minutes until all material was dissolved. To this solution was added, dropwise, a solution of *t*-butyldiphenylsilyl chloride, 2 (0.55 mol, 150.3 g) in 110 Ml of anhydrous THF. During the addition, the reaction mixture was kept at 5 to 10 °C. The addition took approximately one hour. The reaction mixture was stirred overnight under N_2. The end of the reaction was observed by the disappearance of the methyl-(*S*)-lactate as monitored by gas chromatography (R_t 0.7 min., 1; 9.0 mins., 2; 10.5 mins., 3). The reaction mixture was cooled with an ice bath before adding 200 mL of water and 200 mL of ether. The product was further extracted with 2 x 150 mL of ether. The organic layers were combined and washed with 2 x 150 mL of water, 2 x 150 mL of ammonium chloride solution until the pH reached 5, dried with magnesium sulfate, filtered by gravity, and concentrated under reduced pressure. The residue was further pumped under high vacuum overnight. A pale yellow oil was

collected (183.6 g, yield greater than 100%; some silanol by-product from excess silylchloride may be responsible for this excess mass). The oil crystallized upon standing as a white waxy solid. NMR, δ (ppm), (100 MHz, $CDCl_3$: 1.10 (s, 9H); 1.38 (d, J = 5 Hz, 3H); 3.58 (s, 3H); 4.29 (q, J = 5 Hz, 1H); 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

Synthesis of O-(*t*-butyldiphenylsilyl)-(L)-lactic acid (4)

To a solution of O-(t-butyldiphenylsilyl-methyl-(L)-lactate, 3 (0.474 mol, 176.7 g) in 400 mL of methanol was added 400 mL of sodium hydroxide solution (1M). The mixture was heated to 60°C for 2.5 hrs. The solution, originally opaque, became clear. Normal phase TLC indicated a complete hydrolysis of the ester (R_f 0.6, 3; 0.05, 4 hexane: ether, 7 : 3). The methanol was evaporated under reduced pressure. The basic aqueous solution was washed with 3 x 300 mL of ether to remove silanol impurities from the previous step. The aqueous solution was then acidified with conc. HCl until the pH reached 2, and then was extracted with 3 x 400 mL of ether. The organic layer was dried with sodium sulfate, filtered by gravity and concentrated under reduced pressure. The residue was dried under high vacuum for several days. A viscous oil was collected which crystallized upon heating and triturating as a white waxy solid, 142.1 g (overall yield: 87%). NMR, δ (ppm), (100 MHz, $CDCl_3$) : 1.10 (s, 9H); 1.38 (d, J = 5Hz, 3H); 4.29 (q, J = 5Hz, 1H); 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

Synthesis of N-acetyl-S-(*t*-butyldiphenylsilyl)-(L)-lactoyl-(L)-cysteine (5)

To a solution of O-(*t*-butyldiphenylsilyl)-(L)-lactic acid (compound 4)

(91.5 mmol, 20.04 g) in 100 mL of anhydrous THF was added, in four portions through a solid addition funnel, carbonyldiimidazole (109 mmol, 17.77 g). The addition of carbonyldiimidazole produced a significant volume of CO_2. The reaction mixture was stirred for 20 minutes until all the bubbling had stopped. N-Acetyl-(L)-cysteine (119 mmol, 19.5 g) was then added through a funnel in two portions while keeping the temperature of the reaction mixture from rising, via a water bath. Then 80 mL of THF was added to the reaction mixture to help dissolve all of the material. The reaction mixture was stirred for two hours (a thin layer chromatogram (TLC) showed complete conversion of the starting material). Half of the solvent was evaporated and 200 mL of water was added to the reaction mixture. The pH was lowered to two with conc. HCl. The product was then extracted with 3 x 250 mL of ethyl acetate. The organic layers were combined, dried over sodium sulfate, filtered by gravity, evaporated under reduced pressure, and pumped under high vacuum overnight. A white solid (42.91 g) was isolated. The TLC of the material showed several polar impurities. The crude product was then loaded on top of a column packed with 600 g of silica for flash chromatography. The polarity of the eluant was increased from hexane:ethyl acetate:acetic acid (68:30:2) to hexane:ethyl acetate:acetic acid (48:50:2). Product 5 was isolated from condensed appropriate fractions in pure form 41.6 g (96%). TLC : eluant (hexane:ethyl acetate:acetic acid; 35:60:5); R_f : 0.91, 4; 0.27, 5, NMR, δ (ppm),(100 MHz, $CDCl_3$: 1.10 (s, 9H); 1.22 (d, J = 5Hz, 3H); 1.98 (s, 3H); 3.32 (d, J = 6 Hz, 2H); 4.35 (q, J = 5 Hz, 1H); 4.70 (q, J = 6 Hz, 1H); 5.6 - 6.0 (br. s); 6.42 (d, J = 6 Hz, 1H) 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

N-acetyl-S-(L)-lactoyl-(L)-cysteine (6)

N-acetyl-S-(O-(t-butyldiphenylsilyl)-(L)-lactoyl)-(L)-cysteine (compound 5, 89.5 mmol, 42.4 g) was dissolved in 150 ml of THF. To this solution was added a commercially available THF solution of tetrabutyl ammonium fluoride, 1M (130 mmol, 130 mL). The reaction mixture was stirred for eight hours at room temperature. Thin layer chromatography indicated a complete conversion (eluant : chloroform:methanol:acetic acid; 85:10:5; R_f : 0.64, 5; 0.18, 6; 0.53, nBu_4N^+). The solvent was evaporated and the resulting orange oil was stirred vigorously into a suspension with 3 x 300 ml of ether to help remove most of the silanol by-product by decantation. The crude oil (59.2 g) was collected, dissolved in minimum dichloromethane, and loaded on top of a column packed with 900 g of silica. The polarity of the eluant was increased from 100% dichloromethane to dichloromethane:methanol:acetic acid (93:5:2). Several fractions containing various amounts of tetrabutylammonium salt were collected and rechromatographed as described above. After four chromatographies, compound 6 was isolated as a glassy solid from the purest fractions. Traces of acetic acid were removed by azeotropic distillation with cyclohexane. The remaining traces of organic solvents were removed by azeotropic distillation with water. The final material showed the presence of traces of tetrabutylammonium salt amounting to less than 2%, 11 g (52%). IR (cm^{-1}): 1725, 1690, 1640. NMR, δ (ppm), (100MHz, acetone d_6) : 1.32 (d, 3H); 1.93 (s, 3H); AB system : 3.12 (dd, 1H) and 3.45 (dd, 1H); 4.30 (q, 1H); 4.68 (m, 1H); 5.4 - 6.2 (br s); 7.42 (d, NH). Elemental analysis: $C_8H_{13}NO_5S$, % theoretical : C, 40.84; H, 5.57; N, 5.96; S, 13.6; % found: C, 41.35; H, 5.77; N, 5.77;

S, 13.24.

Example 2 S-(N-Acetyl-(L)-cysteine) Derivative of Retinoic Acid

S-(N-Acetyl-(L)-cysteine) derivative of retinoic acid (compound 3)

All trans-retinoic acid, 1 (Aldrich, 11.9 mmol, 3.58 g) was transferred into a dried flask wrapped in dark plastic cover to protect it from light. Under nitrogen, 1 was dissolved in 60 mL of anhydrous THF. To this solution was added a solution of carbonyldiimidazole (15 mmol, 2.44 g) in 20 mL of anhydrous THF. The reaction mixture was stirred under nitrogen for 2.5 hours. The formation of the imidazolide 2 was followed on thin layer chromatography (hexane:ethyl acetate:acetic acid (70:25:5); R_f : 0.49, 1, 0.38, 2). When all the retinoic acid had been transformed to the imidazolide 2, N-acetyl-(L)-cysteine (17.36 mmol, 2.83 g) was added as a solid through a funnel under N_2. The reaction mixture was stirred for 24 hours under N_2. The solvent was evaporated under vacuum at room temperature. The residue was dissolved in 200 mL of ethyl acetate and 120 mL of 0.1 N HCl was added to neutralize the imidazole released by the reaction. The aqueous phase was further extracted with 2 x 100 mL of ethyl acetate. The organic layers were combined, washed with 2 x 100 mL 10% NH_4Cl, dried over magnesium sulfate, filtered by gravity, and evaporated. A crude bright orange material was isolated (5.66 g), which was contaminated by traces of retinoic acid and some unknown polar compound (as seen on thin layer chromatography). The crude material was dissolved in minimum ethyl acetate and loaded on top of a column packed with thirty times the weight of crude

product of silica in hexane:acetic acid (98:2). The polarity of the solvent was increased from hexane:acetic acid (98:2), to hexane:ethyl acetate:acetic acid (48:50:2). All the fractions were collected in the dark. Product 3 was eluted with a solvent polarity ranging from hexane:ethyl acetate:acetic acid (68:30:2) to hexane:ethyl acetate:acetic acid (48:50:2). The pure fractions containing 3 were combined and washed with 3 x 300 mL of water (until the pH reached 5) followed by 300 mL of brine, and then dried over sodium sulfate, and filtered by gravity. The solvent was evaporated under high vacuum with bath temperature not exceeding 30°C in a flask protected from light. NMR indicated a 1:1 mixture with ethyl acetate. This residual solvent could not be removed without heating above 30°C, and above this temperature, compound 3 decomposed.

Example 3 Synthesis of S-oleoyl-N-acetylcysteine

To a dry 1000 mL, 3-neck, 24/40 round bottom flask equipped with an addition funnel, N_2 inlet, and magnetic stir bar, N-acetyl-L-cysteine (Aldrich, 8.77 g, 53.7 mmol) was dissolved in 400 mL of dry tetrahydrofuran (THF, stored over molecular sieves-3A). Triethylamine (Aldrich, 5.73 g, 56.6 mmol) was added, and the reaction mixture chilled in an ice bath. Oleoyl chloride (Nu-Chek-Prep, 17.0 g, 56.4 mmol) was dissolved in 100 mL of THF and placed in the addition funnel. This solution was added dropwise over 30 minutes. A white solid precipitated from the solution. The ice bath was allowed to melt, and the resulting suspension stirred at 25°C for 3 hours, by which time TLC (eluted with a mixture of ethyl acetate and acetic acid) showed the reaction was nearly complete. The reaction mixture was poured into 1.0

liter of ethyl acetate and 0.5 liter of H_2O. The layers were separated, and the organic phase dried over $MgSO_4$, filtered, and concentrated to give an oil (approximately 25 g). This oil was combined with 1.3 g of nearly pure material from an earlier run and chromatographed on 370 g of silica gel, and then eluted with 75% EtOAc/Hexanes with 2% HOAc. Along with pure fractions, several impure fractions were obtained (4.8 g) which were repurified on 100 g of silica gel in the same eluent. All pure fractions were then combined, and concentrated (cyclohexane was used to azeotrope the acetic acid), and then pumped on for 4 days at 25°C, to constant weight. There was obtained 12.08 g (47%) of 1 as a waxy off-white solid, homogeneous on TLC (10% MeOH/EtOAc, R_f= 0.42), mp 94-96°C. Sample spectra are enclosed as well as results of an elemental analysis of the final product.

VI. Pharmaceutical Compositions

Any host that can be treated with a selected biologically active agent can be treated instead with that agent in the form of a thioester or thioether prodrug in a manner that facilitates transport through or targets the relevant biological membrane, as described in detail herein. In particular, humans, equine, canine, bovine and other animals, and in particular, mammals, can be treated by delivery of an effective amount of the thioester or thioether prodrug of a biologically active agent, optionally in a pharmaceutically acceptable carrier or diluent.

The prodrug of the active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of the active agent for the targeted indication without

causing serious toxic effects in the patient treated. If the prodrug exhibits activity in itself, the effective dosage can be estimated using the weight of the derivative, or by other means known to those skilled in the art.

As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes that retain the desired biological activity of the above-identified compounds and exhibit minimal undesired toxicological effects. Pharmaceutically acceptable carboxylic acid and mercaptyl salts are known to those skilled in the art, including inorganic salts with cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with a cation formed with a nitrogenous base such as ammonia, N,N-dibenzylethylene-diamine, D-glucosamine, or ethylenediamine.

The thioester prodrugs disclosed herein are either active in the prodrug form or are cleaved *in vivo* to provide the parent biologically active molecule and the inert or biologically active carrier. Modifications of the active compound and the carrier can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species through the relevant membrane. For example, it is well known in the art that various modifications of the active molecule can effect water and lipid solubility and thus alter the potential for crossing the relevant biological membrane or membranes such as the skin, blood-brain barrier, or cornea. Further, the modifications can affect the bioactivity of the compound, in some cases increasing the activity over the parent compound or increasing the permeability of the parent compound

through the membrane. This can easily be assessed by preparing the derivative and testing its activity according to the methods described herein, or other method known to those skilled in the art.

Solutions or suspensions used for parenteral, intra-dermal, subcutaneous administration can include, for example, the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; anti-bacterial agents such as benzyl alcohol or methyl parabens; anti-oxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

The concentration of prodrug in the pharmaceutical composition will depend on absorption, distribution, deactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. Dosage values will also vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The active ingredient can be administered at once, or can be divided into a number of smaller doses to be administered at varying time intervals.

The prodrug of the active compound or pharmaceutically acceptable derivatives or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action,

such as antibiotics, anti-fungals, anti-inflammatories, disinfectants, or anti-viral compounds.

One preferred mode of administration is oral. Oral compositions will generally include an inert diluent or an edible carrier. The composition can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents. The prodrug can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and

flavors.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any components typically used in such formulations, for example, a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid, sodium bisulfite or Vitamin E; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

Another preferred mode of administration is topical. A pharmaceutically acceptable topical composition containing a prodrug with an effective amount of the biologically active agent is administered for a sufficient time period to alleviate the undesired symptoms and the clinical signs associated with the condition being treated. The concentration of active compound in the drug composition will depend on the permeation rate through the biological membrane, absorption, inactivation, and other factors known to those of skill in the art. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

Suitable vehicles or carriers for topical

application are known, and include lotions, suspensions, ointments, creams, gels, tinctures, sprays, powders, pastes, slow-release transdermal patches, aerosols for asthma, suppositories for application to rectal, vaginal, nasal or oral muçosa, mouthwashes, or swish and spit preparations.

Thickening agents, emollients, and stabilizers can be used to prepare topical compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and ointments are commercially available, especially for ophthalmic and dermatologic applications.

Natural or artificial flavorings or sweeteners can be added to enhance the taste of topical preparations applied for local effect to mucosal surfaces. Inert dyes or colors can be added, particularly in the case of preparations designed for application to oral mucosal surfaces.

The prodrug can be applied in a time release formulation via patches or by slow release polymers. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, ccllagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. The materials can also be obtained commercially from Alza

Corporation.

When used for ocular therapy, the prodrug can be administered in any convenient manner, including in an inert vehicle to eye tissue by intraocular injection or topically. The term "inert vehicle" refers to any vehicle that is inert to both the prodrug and to the host, and can includes adjuvants, preservatives, buffers, and demulcents. As used herein, "ophthalmically effective amount" is that amount which in the composition administered and by the technique administered, provides an amount of therapeutic agent to the involved eye tissues sufficient to improve visual function or prevent or minimize its loss for a desired period of time.

When the intraocular injection is subconjunctival, an ophthalmically effective amount of prodrug can be, for example, administered typically in a polymeric carrier such as a dextran or polysorbate 80, which optionally contains additives such as disodium edetate, sodium sulfite, and/or sodium chloride, and sodium hydroxide or hydrogen chloride for pH adjustment. When the intraocular injection is intracameral or intravitreal, an effective amount of the prodrug is typically administered in a vehicle containing phosphate buffered saline, citrate buffered saline, or chrondrotin sulfate, or in a polymeric vehicle such as sodium hyaluronate, or hyaluronic acid, purified polyacrylamide or polysorbate 80, with the formulation containing sodium hydroxide or hydrogen chloride for pH adjustment.

When the ocular administration is topical, a topical formulation containing an effective amount of the prodrug derivative is administered in a topically acceptable carrier. One example is an aqueous polymeric solution,

aqueous suspension, ointment, gel or cream vehicle. Except for ointments, these vehicles may contain liposomes for creating a reservoir of dissolved agent for contact with the tear film.

Topical ocular administration is preferable when the target of the treatment is located in or near the anterior chamber of the eye. By contrast, because the flow of aqueous humor is from the ciliary body (behind the iris) forward towards the cornea before it exits through the trabecular meshwork and Schlemm's canal, penetration of drugs to the back of the eye when administered topically to the front of the eye occurs with difficulty. It is therefore often more effective to administer drugs intended for the treatment of uveal and retinal diseases by the systemic route where access to the eye occurs through the choroid plexus, or by the intravitreal route. Some of the more severe eye diseases affect those targets which are difficult to treat effectively by the topical route and they can be associated with markedly impaired vision or blindness. Accordingly, the topical route is preferred to convenience of individual patient self-administration, and the intraocular and systemic routes are preferred for surgical and presurgical administration.

In order to maintain an ocularly adequate therapeutic level of drug in the back of the eye where surgery is not involved, or has been concluded, the present invention also contemplates the treatment of an ophthalmic disease by administration of a therapeutically effective amount of the prodrug in a suitable carrier, by oral, intramuscular and intravenous routes, in addition to the convenient topical route or by intraocular injection. To administer the

intravenous formulation for treatment of the eye, the drug formulations are preferably dose injected of infused into a major vein (e.g., in the arm are), or introduced by continuous intravenous drip.

Intramuscular formulations will typically include an effective amount of the prodrug in an aqueous solution or suspension for intramuscular delivery, and can include, for example, polysorbate 80, methyl cellulose, and other demulcents. Other additives desirably added to intramuscular formulations include sodium chloride and sodium bisulfite. To administer the intramuscular formulations for treatment of the eye, the drug formulations will be injected for example into the upper outer quadrant of the gluteal muscle.

Modifications and variations of the present invention will be obvious to those skilled in the art from the foregoing detailed description of the invention. Such modifications and variations are intended to come within the scope of the appended claims.

We claim.

1. A method for the delivery of a biologically active agent through a biological membrane, comprising providing the biologically active agent as a compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R' is an inert or biologically active molecule which enhances the lipophilic nature of the resulting compound or serves to target the compound to a particular membrane, cell type or cell component.

2. The method of claim 1, wherein R' is selected from the group consisting of alkyl, alkenyl, alkynyl, alkyaryl, aralkyl, haloalkyl, haloalkenyl, haloalkynyl, -C_{1-10}alkyl(oxy)C_{1-10}alkyl, -C_{1-10}alkyl(thio)C_{1-10}alkyl, aryloxyalkyl, phenyl, benzyl, pyrryl, furyl, pyridyl, thiophenyl, pyrimidyl, thienyl, isothiazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, purinyl, carbozolyl, isoxazolyl, and naphthyl.

3. The method of claim 1, wherein R'C(O)- is the residue of a saturated or unsaturated fatty acid.

4. The method of claim 3, wherein the fatty acid is selected from the group consisting of lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic and petriselinic acid.

5. The method of claim 1, wherein R' is

selected from the group consisting of the residue of retinoic acid.

6. The method of claim 1, wherein R is the residue of minoxidil.

7. The method of claim 1, wherein the compound is $CH_3C(O)NC(H)C(O)OR"CH_2SR'$, wherein R" is hydrogen, alkyl, aryl, alkaryl, aralkyl, alkyloxyalkyl, aryloxyalkyl, or an inorganic cation, including but not limited to sodium, potassium, magnesium, calcium, zinc, bismuth, barium, aluminum, copper, cobalt, nickel, and cadmium.

8. The method of claim 1, wherein R' is also biologically active.

9. The method of claim 8, wherein R' is the residue of a compound selected from the group consisting of vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, 1α,25-dihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3), vitamin E, and vitamin C.

10. The method of claim 1, wherein R or R' is selected from the group consisting of erythromycin, propionylerythromycin, neomycin, gentomycin, mechlocyclin, tobramycin, and kanamycin.

11. The method of claim 1, wherein R is the residue of a drug.

12. The method of claim 11, wherein the drug is selected from the group consisting of an immunosuppressant, an antioxidant, an anesthetic, a chemotherapeutic agent, a steroid, a hormone, an antibiotic, an antiviral, an antifungal, an antiproliferative, an antihistamine, an anticoagulant, an antiphotoaging agent, a melanotropic peptide, and a nonsteroidal or steroidal anti-inflammatory.

13. The method of claim 1, wherein the biologically active agent is selected from the group consisting of a nucleoside, nucleotide, oligonucleotide, cDNA, a nucleic acid, and a gene.

14. The method of claim 1, wherein the biologically active agent is selected from the group consisting of a protein, polysaccharide, nucleoprotein, lipoprotein, synthetic polypeptide, or a small molecule linked to a protein, carbohydrate, glycoprotein, steroid, nucleic acid, lipid, or combination thereof.

15. The method of claim 1, wherein the biologically active agent is an antisense oligonucleotide.

16. The method of claim 1, wherein R' is a moiety that targets the compound to a specific membrane, cell type or cell component.

17. The method of claim 16, wherein R' is the residue of a steroid.

18. The method of claim 16, wherein R' is the residue of a compound selected from the group consisting of a hormone, a hormone receptor, a cell specific receptor, a ligand that binds to a cell specific receptor, an antibody, an antibody fragment, an antigen, and a T-cell receptor fragment.

19. The method of claim 17, wherein the steroid receptor of a cancer cell.

20. A pharmaceutical composition for the delivery of a biologically active agent through a biological membrane, comprising an effective amount of the biologically active agent modified as a compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R' is an inert or biologically active molecule which enhances the lipophilic nature of the resulting compound or

serves to target the compound to a particular membrane, cell type or cell component.

21. The composition of claim 20, wherein R' is selected from the group consisting of alkyl, alkenyl, alkynyl, alkyaryl, aralkyl, haloalkyl, haloalkenyl, haloalkynyl, -C_{1-10}alkyl(oxy)C_{1-10}alkyl, -C_{1-10}alkyl(thio)C_{1-10}alkyl, aryloxyalkyl, phenyl, benzyl, pyrryl, furyl, pyridyl, thiophenyl, pyrimidyl, thienyl, isothiazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, purinyl, carbozolyl, isoxazolyl, and naphthyl.

22. The composition of claim 20, wherein R'C(O)- is the residue of a saturated or unsaturated fatty acid.

23. The composition of claim 20, wherein the fatty acid is selected from the group consisting of lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic and petriselinic acid.

24. The composition of claim 20, wherein R' is selected from the group consisting of the residue of retinoic acid.

25. The composition of claim 20, wherein R is the residue of minoxidil.

26. The composition of claim 20, wherein the compound is $CH_3C(O)NC(H)C(O)OR"CH_2SR'$, wherein R" is hydrogen, alkyl, aryl, alkaryl, aralkyl, alkyloxyalkyl, aryloxyalkyl, or an inorganic cation, including but not limited to sodium,

potassium, magnesium, calcium, zinc, bismuth, barium, aluminum, copper, cobalt, nickel, and cadmium.

27. The composition of claim 20, wherein R' is also biologically active.

28. The composition of claim 20, wherein R' is the residue of a compound selected from the group consisting of vitamin D_1, vitamin D_2, vitamin D_3, vitamin D_4, 1α,25-dihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3, vitamin E, and vitamin C.

29. The composition of claim 20, wherein R or R' is selected from the group consisting of erythromycin, propionylerythromycin, neomycin, gentomycin, mechlocyclin, tobramycin, and kanamycin.

30. The composition of claim 20, wherein R is the residue of a drug.

31. The composition of claim 20, wherein the drug is selected from the group consisting of an immunosuppressant, an antioxidant, an anesthetic, a chemotherapeutic agent, a steroid, a hormone, an antibiotic, an antiviral, an antifungal, an antiproliferative, an antihistamine, an anticoagulant, an antiphotoaging agent, a melanotropic peptide, and a nonsteroidal or steroidal anti-inflammatory.

32. The composition of claim 20, wherein the biologically active agent is selected from the group consisting of a nucleoside, nucleotide, oligonucleotide, cDNA, a nucleic acid, and a gene.

33. The composition of claim 20, wherein the biologically active agent is selected from the group consisting of a protein, polysaccharide, nucleoprotein, lipoprotein, synthetic polypeptide, or a small molecule linked to a protein,

carbohydrate, glycoprotein, steroid, nucleic acid, lipid, or combination thereof.

34. The composition of claim 20, wherein the biologically active agent is an antisense oligonucleotide.

35. The composition of claim 20, wherein R' is a moiety that targets the compound to a specific membrane.

36. The composition of claim 20, wherein R' is the residue of a steroid.

37. The composition of claim 35, wherein R' is the residue of a compound selected from the group consisting of a hormone, a hormone receptor, a cell specific receptor, a ligands that binds to a cell specific receptor, an antibody, an antibody fragment, an antigen, and a T-cell receptor fragment.

38. The composition of claim 34, wherein the steroid binds to the membrane of a cancer cell.

39. A compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R'H is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of an immunosuppressant, an antioxidant, an anesthetic, a chemotherapeutic agent, a steroid, a hormone, an antibiotic, an antiviral, an antifungal, an antiproliferative, an antihistamine, an anticoagulant, an antiphotoaging agent, a melanotropic peptide, and a nonsteroidal or steroidal anti-inflammatory.

40. A compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R'H is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of a nucleoside, nucleotide,

oligonucleotide, cDNA, a nucleic acid, and a gene.

41. A compound selected from the group consisting of R-C(O)S-R' and R-SC(O)-R', wherein R is the residue of the biologically active agent and R' is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of a protein, polysaccharide, nucleoprotein, lipoprotein, synthetic polypeptide, or a small molecule linked to a protein, carbohydrate, glycoprotein, steroid, lipid, or combination thereof.

42. A method for the delivery of a biologically active agent through a biological membrane, comprising providing the biologically active agent of the formula R-S-R', wherein R is the residue of the biologically active agent and R' is a lipophilic moiety.

43. A pharmaceutical composition for the delivery of a biologically active agent through a biological membrane, comprising an effective amount of the biologically active agent modified as a compound of the formula R-S-R', wherein R is the residue of the biologically active agent and R' is a lipophilic moiety.

44. A compound of the formula R-S-R', wherein R is the residue of the biologically active agent and R' is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of an immunosuppressant, an antioxidant, an anesthetic, a chemotherapeutic agent, a steroid, a hormone, an antibiotic, an antiviral, an antifungal, an antiproliferative, an antihistamine, an anticoagulant, an antiphotoaging agent, a melanotropic peptide, and a nonsteroidal or steroidal anti-inflammatory.

45. A compound of the formula R-S-R', wherein R is the residue of the biologically active

agent and R' is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of a nucleoside, nucleotide, oligonucleotide, cDNA, a nucleic acid, and a gene.

46. A compound of the formula R-S-R', wherein R is the residue of the biologically active agent and R' is a lipophilic moiety, wherein the biologically active molecule is selected from the group consisting of a protein, polysaccharide, nucleoprotein, lipoprotein, synthetic polypeptide, or a small molecule linked to a protein, carbohydrate, glycoprotein, steroid, lipid, or combination thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04084

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/07, 31/59, 37/00; C07K 1/00; C07H 15/24, 19/00, 21/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 29, 39, 40, 41, 167, 725; 530/350, 395; 536/7.2, 22; 552/502; 436/71; 514/43, 44; 536/1.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 3,980,631 (PROCHAZKA ET AL) 14 September 1976, col. 2, lines 18-46.	1-46
A	US, A, 4,286,964 (SEED) 01 September 1981, col. 2, lines 23-44.	1-46

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JUNE 1995	Date of mailing of the international search report 30 JUN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELLI PESELEV Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04084

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

436/71; 514/2, 29, 39, 40, 41, 43, 44, 167, 725; 530/350, 395; 536/1.1, 7.2, 22; 552/502